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10/593,016	09/14/2006	Thaddeus C. George	BIOL0123	5733

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/593,016	GEORGE ET AL.	
	Examiner	Art Unit	
	Jason Heidemann	2624	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 July 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-24 and 27-44* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-24 and 27-44* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *12 June 2008* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *11/10/2010*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. This is the first Office action on the merits of Application No. 10/593016 filed on 09/14/2006. Claims 1-24, 27- 44 are presented for examination. Claims 1-24, 27-44 are rejected for the reasons indicated hereinbelow.

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after issue of the application. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the application from issue has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 07/20/2010 has been entered.

Response to Amendment and Arguments

The amendment received 07/20/2010 has been entered in full.

I. Amendments

Applicant has amended each independent, as shown below:

For claims 1, 8, 16,

"A method for identifying a specific cell, to enable a determination to be made as to whether the specific cell corresponds to a known cell type, wherein the known cell type relates to the viability of the cell, comprising the steps of: ..."

For Claim 27

"A kit for use in a multispectral imaging system to identify a specific cell, comprising a single nuclear marker, wherein a cell is contacted with the single nuclear marker for a time sufficient to allow identification of the cell as an apoptotic cell or a necrotic cell with the multispectral imaging system using only a single nuclear marker."

II. Response to 35 U.S.C. § 102(b) arguments

Applicant argues with respect to the 102(b) rejections of Claims 27 and 28 as being anticipated by U.S. Pub. Application No. 2002/0159625 (Elling)

- Elling does not teach or suggest the use of a single nuclear marker to identify whether a cell is an apoptotic cell or a necrotic cell.
- Elling does not teach or suggest that any of these reagents are used to produce images for the purpose of identifying a cell as an apoptotic cell or as a necrotic cell.
- The fact that one of the reagents listed by Elling is the same as recited in Claim 28 is not determinative of Elling teaching that function, since reagents can be used to label a cell nucleus for a number of different purposes not related to

identifying a cell as apoptotic or necrotic, and Elling does not teach or suggest the use of 7-aminoactinomycin D for use in identifying a cell as an apoptotic cell or a necrotic cell.

Examiner reminds applicant that Claim 27 and 28 are directed towards a kit, which is an article claim (MPEP 2112.01), which is composed of a single element/article, "a single nuclear marker". An article claim is defined by its structure that is the claim only recites a structure of "a single nuclear marker". A prior art element *does not* become patentable to the applicant because they claim to have discovered a further advantage of the element suggested in the prior art see MPEP § 2112. The wherein cause describes the intended use of the element in the kit, that is "a cell is contacted with the single nuclear marker for a time sufficient to allow identification of the cell as an apoptotic cell or a necrotic cell with the multispectral imaging system using only a single nuclear marker". Examiner states any recitation directed to the manner in which a claimed kit is intended to be used does not distinguish the claimed kit from the prior art - if the prior art has the capability to so perform (2114). Examiner believes for Claim 27 to be considered allowable over the prior art it must structurally distinguishable from the prior art, and the intended use is insufficient to prove so. Further, Elling as admitted by applicant is the same element, thus the prior art is identical to the present invention, and the function is inherent or intrinsic to that element, that is the ability to "allow identification of the cell as an apoptotic cell or a necrotic cell with the multispectral imaging system using only a single nuclear marker". Examiner requests applicant to provide sufficient evidence that the prior art, Elling, does not posses this

characteristic (MPEP 2112). The examiner maintains the rejection of Claim 27 and 28 over the prior art of record.

III. Response to 35 U.S.C. § 103(a) arguments

Applicant's arguments with respect to art rejections to claims the pending claims have been considered but are moot in view of the new ground(s) of rejection due to the amendments filed by the Applicant(s).

The Examiner believes that all the arguments of the Applicant have been properly addressed and explained.

Priority

This application claims benefit of a National Stage Application No. PCT/US05/08870, filed 03/16/2005.

This application claims benefit of an earlier filing date under 35 U.S.C. 119(e) of U.S. Provisional Application 60/553502, filed 16 March 2004.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on 11/10/2010 is in compliance with the provisions of 37 C.F.R. § 1.97. Accordingly, the examiner has considered all references cited in the submitted IDS(s).

Drawings

The subject matter of this application admits of illustration by a drawing to facilitate understanding of the invention. Applicant is required to furnish a drawing under 37 CFR 1.81(c). No new matter may be introduced in the required drawing. Each drawing sheet submitted after the filing date of an application must be labeled in the top margin as either "Replacement Sheet" or "New Sheet" pursuant to 37 CFR 1.121(d).

The drawings are objected to under 37 CFR 1.83(a). The drawings must show every feature of the invention specified in the claims. Claim 43 is not shown in the drawings. Therefore, the features of claim 43, "when no **blebbing** is determined to be present by analyzing the brightfield image, and no nuclear marker is determined to be present in the cellular nucleus by analyzing the fluorescent image, it can be concluded that the specific cell is the viable cell type; when **blebbing** is determined to be present by analyzing the brightfield image, and no nuclear marker is determined to be present in the cellular nucleus by analyzing the fluorescent image, it can be concluded that the specific cell is the type of cell in a relatively early stage of apoptosis; when **blebbing** is

determined to be present by analyzing the brightfield image, and the nuclear marker is determined to be present in the cellular nucleus by analyzing the fluorescent image, it can be concluded that the specific cell is the type of cell in a relatively late stage of apoptosis; and when no **blebbing** is determined to be present by analyzing the brightfield image, and the nuclear marker is determined to be present in the cellular nucleus by analyzing the fluorescent image, it can be concluded that the specific cell is of the necrotic type of cell" must be shown or the feature(s) canceled from the claim(s). No new matter should be entered.

Corrected drawing sheets in compliance with 37 CFR 1.121(d) are required in reply to the Office action to avoid abandonment of the application. Any amended replacement drawing sheet should include all of the figures appearing on the immediate prior version of the sheet, even if only one figure is being amended. The figure or figure number of an amended drawing should not be labeled as "amended." If a drawing figure is to be canceled, the appropriate figure must be removed from the replacement sheet, and where necessary, the remaining figures must be renumbered and appropriate changes made to the brief description of the several views of the drawings for consistency. Additional replacement sheets may be necessary to show the renumbering of the remaining figures. Each drawing sheet submitted after the filing date of an application must be labeled in the top margin as either "Replacement Sheet" or "New Sheet" pursuant to 37 CFR 1.121(d). If the changes are not accepted by the examiner, the applicant will be notified and informed of any required corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

Examiner's Note

Examiner has cited particular columns and line numbers or figures in the references as applied to the claims below for the convenience of the applicant. Although the specified citations are representative of the teachings in the art and are applied to the specific limitations within the individual claim, other passages and figures may apply as well. It is respectfully requested from the applicant, in preparing the responses, to fully consider the references in entirety as potentially teaching all or part of the claimed invention, as well as the context of the passage as taught by the prior art or disclosed by the examiner.

1. Claim Interpretation

Examiner notes viable and viability were never recited explicitly in the specification. Examiner has assigned the meaning to viable cell to refer to living cell as recited in the specification. Examiner has assigned the meaning to viability of the cell, to be determining whether the cell is living or dead, as recited in the specification.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 30-44, are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The term "relatively" is a relative term which renders the claim indefinite. The term "relatively" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. For example, what are the metes and bounds of "relatively larger" as found in claim 30? What are the metes and bounds of "relatively early" as found in claim 44? Any amendment to the claims must be commensurate in scope with the corresponding disclosure. See MPEP § 2173.03, 2173.05.

Further, Claim 43, uses the phrase "**can be**" which renders the claim indefinite because it is unclear whether the limitation(s) following the phrase are part of the claimed invention. See MPEP § 2173.03, 2173.05.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public

use or on sale in this country, more than one year prior to the date of application for patent in the United States.

(e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

A.) Claims 27 and 28 are rejected under 35 U.S.C. 102(b) as being anticipated over Elling (US PgPub # 2002/0159625, hereinafter Elling). Elling is filed in applicant's filed IDS on 3/11/2010.

As to Claim 27, Elling discloses a kit for use in a multispectral imaging system to identify a specific cell, comprising

a single nuclear marker (Elling, Fig.1, [0010], [0014], [0043], [0076], discloses using a single stain, and the stain is used to identify living and fixed (death) cells),

wherein a cell is contacted with the single nuclear marker for a time sufficient to allow identification of the cell as an apoptotic cell or a necrotic cell with the multispectral imaging system using only a single nuclear marker (Elling, Fig.1, [0010], [0014], [0043], [0076], discloses using a single stain, and the stain is used to identify living and fixed cells, while Elling doesn't explicitly disclose using the single nuclear marker for identification of the cell as an apoptotic cell or a necrotic cell , the wherein cause describes only an intended use of the element in the kit, further the prior art has the capability to so perform the intended use since it is comprised of the same element,

given the prior art is identical to the present invention the function is inherent or intrinsic to that element (MPEP 2112)).

As to Claim 28, Elling discloses the kit of claim 27 wherein the single nuclear marker is 7-aminoactinomycin D (Elling, [0076], 7-aminoactinomycin D is used to label (identify) the nucleus of living and fixed cells).

B.) Claim 27 is further rejected and Claim 29 is rejected under 35 U.S.C. 102(e) as being anticipated over Ortyn et al. (US PGPub # 20040021868, hereinafter Ortyn).

As to Claim 27, Ortyn discloses a kit for use in a multispectral imaging system to identify a specific cell, comprising a single nuclear marker (**Ortyn, [0121], "cell nuclei stained with a green fluorescent dye" (nuclear marker)**), wherein a cell is contacted with the single nuclear marker for a time sufficient to allow identification of the cell as an apoptotic cell or a necrotic cell with the multispectral imaging system using only a single nuclear marker (**Ortyn, abstract, [0014], [0135], identify "cell viability and apoptosis staging, and necrosis differentiation"; [0136], "cell analysis and classification"; [0138], "cell identification", note: the wherein cause describes only an intended use of the element in the kit, further the prior art has the capability to so perform the intended use since it is comprised of the same**

element, given the prior art is identical to the present invention the function is inherent or intrinsic to that element (MPEP 2112)).

As to Claim 29, Ortyn teaches a method for identifying a specific cell, to determine a type of the specific cell, (**Ortyn, abstract, [0016], [0135], [0138], method for determining characteristics of a moving object, can be used in "cell identification", "necrosis differentiation", "cell viability"**) comprising the steps of:

exposing the specific cell to a nuclear marker that will bind to DNA in a nucleus of the cell (**Ortyn, [0121], "cell nuclei stained with a green fluorescent dye"**);

collecting spatial frequency image data of the specific cell in which the nuclear marker is present (**Ortyn, fig. 5, [0080], [0121] uses a TDI detector, el 44, to obtain an image of the cell**); and

analyzing the spatial frequency image data to determine a type of the specific cell, wherein the type of the specific cell is determined by a condition of material in a nucleus of the specific cell, as indicated by the spatial frequency image data (**Ortyn, [0063], "spatial frequency content", [0135], identify "cell viability and apoptosis staging, and necrosis differentiation"; [0136], "cell analysis and classification"; [0138], "cell identification"**).

Claim Rejections - 35 USC § 103

2. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all

obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

A.) Claims 1-23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ortyn in view of Young et al. ("Towards automatic cell identification in DIC microscopy", November 1998, Journal of Microscopy, Vol. 192, Pt 2, pp. 186–193., hereinafter Young)

As to Claim 1, Ortyn teaches a method for identifying a specific cell, to enable a determination to be made as to whether the specific cell corresponds to a known cell type, wherein the known cell type relates to the viability of the cell (**Ortyn, abstract, [0016], [0135], [0138], method for determining characteristics of a moving object, can be used in "cell identification", "necrosis differentiation"**), comprising the steps of:

providing spatial frequency content data from a side scatter image of the known cell type (**Ortyn, fig. 5; [0063], [0078-0080], "collection lens 32 is at about 90" angle relative to the directions of the light incident"; [0135], "dark field"**);

directing incident light at the specific cell, using a detector to obtain the side scatter image of the specific cell (**Ortyn, fig. 5; [0078-0080]**); and

using spatial frequency content of the side scatter image of the specific cell to determine if the specific cell corresponds to the known cell type (**Ortyn, [0063], "spatial frequency content", [0135], identify "cell viability and apoptosis staging, and necrosis differentiation"; [0136], "cell analysis and classification"; [0138], "cell identification"**). However, Ortyn doesn't explicitly disclose a comparing of data to previous collected data, as required by the limitation "comparing the spatial frequency content of the side scatter image of the specific cell to the spatial frequency content data of the side scatter image of the known cell type to determine if the specific cell corresponds to the known cell type."

Young teaches a general method for cell identification based on template matching (cell comparison), where the templates are constructed from a known cell types (**Young, abstract, page 192, section 4. discussion**). Further, Young motivates this method by demonstrated its ability to automatically identify and measure individual cells in clusters (**Young, abstract**). Young's "Template matching" is a method for automating the identification and measuring of cells in microscope image, where correspondences are found between a template sub-image and the full image, based on a goodness-of-fit statistic evaluated at all possible positions, where Young utilizes FFTs in order to reduce computer time to an acceptable level (**Young, abstract, page 187, left col., paragraph 3**).

Hence the prior art includes each element claimed, although not necessarily in a single prior art reference, with the only difference between the claimed invention and the prior art being the lack of actual combination of the elements in a single prior art reference

Ortyn & Young are combinable because they are from the imaging arts of cell analysis and identification. Thus, It would have been obvious to one of ordinary skilled in the art at the time of inventions looking at the teachings and suggestions of Ortyn to provide an improved method of cell analysis in view of Young's disclosure to include the methodology of Template matching which includes comparison of the collected data to a previous collected data of a known type (template). The modification to Ortyn, could be accomplished by including a template matching algorithm, which allows the comparison of a template, previous collected data of a known type (template), to newly acquired image data to identify the cell. Further a person of ordinary skill in the art would have recognized the compatibility of template matching with the method of Ortyn. The combination has a reasonable expectation of success in that the modifications can be made using conventional and well known engineering and/or programming techniques, the template matching as taught by Young is not altered and continues to perform the same function as separately, and the resultant combination produces the highly predictable result of "comparing the spatial frequency content of the side scatter image of the specific cell to the spatial frequency content data of the side scatter image of the known cell type to determine if the specific cell corresponds to the known cell type." One of ordinary skilled in the art would have been motivated to combine the

teachings of Ortyn to the system/apparatus of Young in order to use Young's "Template matching" for automating the identification and measuring of cells in microscope image, since it utilizes FFTs in order to reduce computer time to an acceptable level.

As to Claim 2, the combination of Ortyn and Young teach the method of claim 1 wherein there is relative motion between the specific cell and the detector (**Ortyn, [0066], [0069], "fluid flow 22"**).

As to Claim 3, the combination of Ortyn and Young teach the method of claim 1 wherein the specific cell identified is contained within a heterogeneous cell population, and side scatter image data is collected for the heterogeneous cell population (**Ortyn, [0135], analyzing tens of thousands of cells, rare cell detection and differentiation, further [0107] allows the analysis of heterogeneous cells see male/female cells**).

As to Claim 4, the combination of Ortyn and Young teach the method of claim 1 wherein the specific cell identified is an apoptotic cell (**Ortyn, [0135], identify apoptosis staging**).

As to Claim 5, the combination of Ortyn and Young teach the method of claim 4 wherein the apoptotic cell is an early stage apoptotic cell or a late stage apoptotic cell (**Ortyn, [0135], identify apoptosis staging**).

As to Claim 6, the combination of Ortyn and Young teach the method of claim 1 wherein the specific cell identified is a necrotic cell (**Ortyn, [0135], identify necrosis differentiation**).

As to Claim 7, the combination of Ortyn and Young teach the method of claim 1 wherein the specific cell identified is at least one of an apoptotic cell and a necrotic cell (**Ortyn, [0135], identify "apoptosis staging**).

As to Claim 8, Ortyn teaches a method for identifying a specific cell, to enable a determination to be made as to whether the specific cell corresponds to a known cell type (**Ortyn, abstract, [0016], [0135], [0138], method for determining characteristics of a moving object, can be used in "cell identification", "necrosis differentiation"**), wherein the known cell type relates to the viability of the cell, comprising the steps of: providing spatial frequency content data from a brightfield image of the known cell type (**Ortyn, fig. 5; [0135], "brightfield", [0063], [0078-0080], "collection lens 32 is at about 90" angle relative to the directions of the light incident"**); directing incident light at the specific cell, using a detector to obtain the brightfield image of the specific cell (**Ortyn, fig. 5; [0078-0080]**); and using spatial frequency content of the side scatter image of the specific cell to determine if the specific cell corresponds to the known cell type (**Ortyn, [0063], "spatial frequency content", [0135], identify "cell viability and apoptosis staging, and necrosis differentiation"; [0136], "cell analysis and classification"; [0138], "cell identification"**). However, Ortyn doesn't explicitly disclose a comparing of data to previous collected data, as required by the limitation "comparing the spatial frequency

content of the side scatter image of the specific cell to the spatial frequency content data of the side scatter image of the known cell type to determine if the specific cell corresponds to the known cell type."

Young teaches a general method for cell identification based on template matching (cell comparison), where the templates are constructed from a known cell types (**Young, abstract, page 192, section 4. discussion**). Further, Young motivates this method by demonstrated its ability to automatically identify and measure individual cells in clusters (**Young, abstract**). Young's "Template matching" is a method for automating the identification and measuring of cells in microscope image, where correspondences are found between a template sub-image and the full image, based on a goodness-of-fit statistic evaluated at all possible positions, where Young utilizes FFTs in order to reduce computer time to an acceptable level (**Young, abstract, page 187, left col., paragraph 3**). See motivation for the combination as cited in claim 1.

As to Claim 9, the combination of Ortyn and Young teach the method of claim 8 wherein there is relative motion between the specific cell and the detector (**Ortyn, [0066], [0069], "fluid flow 22"**).

As to Claim 10, the combination of Ortyn and Young teach the method of claim 8 wherein the specific cell identified is contained within a heterogeneous cell population, and brightfield image data is collected for the heterogeneous cell population (**Ortyn, [0135], analyzing tens of thousands of cells, rare cell detection and**

differentiation, further [0107] allows the analysis of heterogeneous cells see male/female cells).

As to Claim 11, the combination of Ortyn and Young teach the method of claim 8 wherein the specific cell identified is an apoptotic cell (**Ortyn, [0135], identify apoptosis staging**).

As to Claim 12, the combination of Ortyn and Young teach the method of claim 11 wherein the apoptotic cell is an early stage apoptotic cell or a late stage apoptotic cell (**Ortyn, [0135], identify apoptosis staging**).

As to Claim 13, the combination of Ortyn and Young teach the method of claim 8 wherein the specific cell identified is a necrotic cell (**Ortyn, [0135], identify necrosis differentiation**).

As to Claim 14, the combination of Ortyn and Young teach the method of claim 8 wherein the specific cell identified is at least one of an apoptotic cell and a necrotic cell (**Ortyn, [0135], identify "apoptosis staging**).

As to Claim 15, the combination of Ortyn and Young teach the method of claim 8 wherein the spatial frequency content is of the nucleus (**Ortyn, [0063], [0107], [0121], Fig.11, the TDI detector distinguishes the spatial potion, images the cell and it's nucleus**);

As to Claim 16, Ortyn teaches A method for identifying a specific cell, to enable a determination to be made as to whether the specific cell corresponds to a known cell

type, wherein the known cell type relates to the viability of the cell, (**Ortyn, abstract, [0016], [0135], [0138], method for determining characteristics of a moving object, can be used in "cell identification", "necrosis differentiation", "cell viability",**) comprising the steps of:

providing an image of the known cell type that has been marked with a nuclear marker (**Ortyn, fig. 5; [0135], "brightfield", [0063], [0078-0080], "collection lens 32 is at about 90" angle relative to the directions of the light incident"**);

providing spatial frequency content data from the image of the known cell type that has been marked with the nuclear marker (**Ortyn, fig. 5; [0135], "brightfield", [0063], [0078-0080], "collection lens 32 is at about 90" angle relative to the directions of the light incident"**);

contacting the specific cell with the nuclear marker (**Ortyn, [0121], "cell nuclei stained with a green fluorescent dye"**);

directing incident light at the marked specific cell (**Ortyn, fig. 5; [0078-0080], uses a light source (see Fig.5, el 66) to excite fluorescence**);

using a detector to obtain an image of the marked specific cell (**Ortyn, fig. 5, [0080], [0121] uses a TDI detector, el 44, to obtain an image of the cell**); and using spatial frequency content of the side scatter image of the specific cell to determine if the specific cell corresponds to the known cell type (**Ortyn, [0063], "spatial**

frequency content", [0135], identify "cell viability and apoptosis staging, and necrosis differentiation"; [0136], "cell analysis and classification"; [0138], "cell identification"). However, Ortyn doesn't explicitly disclose a comparing of data to previous collected data, as required by the limitation "comparing the spatial frequency content of the side scatter image of the specific cell to the spatial frequency content data of the side scatter image of the known cell type to determine if the specific cell corresponds to the known cell type."

Young teaches a general method for cell identification based on template matching (cell comparison), where the templates are constructed from a known cell types (**Young, abstract, page 192, section 4. discussion**). Further, Young motivates this method by demonstrated its ability to automatically identify and measure individual cells in clusters (**Young, abstract**). Young's "Template matching" is a method for automating the identification and measuring of cells in microscope image, where correspondences are found between a template sub-image and the full image, based on a goodness-of-fit statistic evaluated at all possible positions, where Young utilizes FFTs in order to reduce computer time to an acceptable level (**Young, abstract, page 187, left col., paragraph 3**). See motivation for the combination as cited in claim 1.

As to Claim 17, the combination of Ortyn and Young teach the method of claim 16 wherein there is relative motion between the specific cell and the detector (**Ortyn, [0066], [0069], "fluid flow 22"**).

As to Claim 18, the combination of Ortyn and Young teach the method of claim 16 wherein the specific cell identified is contained within a heterogeneous cell

population, and image data is collected for the heterogeneous cell population (**Ortyn, [0135], analyzing tens of thousands of cells, rare cell detection and differentiation, further [0107] allows the analysis of heterogeneous cells see male/female cells**).

As to Claim 19, the combination of Ortyn and Young teach the method of claim 16 wherein the specific cell identified is an apoptotic cell (**Ortyn, [0135], identify apoptosis staging**).

As to Claim 20, the combination of Ortyn and Young teach the method of claim 19 wherein the apoptotic cell is an early stage apoptotic cell or a late stage apoptotic cell (**Ortyn, [0135], identify apoptosis staging**).

As to Claim 21, the combination of Ortyn and Young teach the method of claim 16 wherein the specific cell identified is a necrotic cell (**Ortyn, [0135], identify necrosis differentiation**).

As to Claim 22, the combination of Ortyn and Young teach the method of claim 16 wherein the specific cell identified is at least one of an apoptotic cell and a necrotic cell (**Ortyn, [0135], identify "apoptosis staging**).

As to Claim 23, the combination of Ortyn and Young teach the method of claim 16 wherein a single nuclear marker is used (**Ortyn, [0121], the cell nuclei is stained with a green fluorescent dye (single nuclear marker)**).

B.) Claim 24 is rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Ortyn and Young as applied above and further in view of Fraatz (US Patent # 5372936, hereinafter Fraatz).

As to Claim 24, the combination of Ortyn and Young teach the method of claim 16. However, Ortyn doesn't explicitly teach wherein the single nuclear marker is 7-aminoactinomycin D.

Fraatz teaches using 7-aminoactinomycin D as a maker for imagining samples (**Fraatz, Column 8, Table 1, Table 2, Column 6, lines 1-20**). Fraatz performs analysis for identifying biological activities in specimens (cells). It would have been obvious to one of ordinary skilled in the art at the time of inventions to modify the method for identifying cells of the combination of Ortyn and Young, by using 7-aminoactinomycin D as the nuclear marker as to the teaching of Fraatz. The combination of Ortyn and Young and Fraatz are analogous in the art of image based biological analysis. One of ordinary skilled in the art would have been motivated to combine the teachings of Fraatz to the method of the combination of Ortyn and Young in order to use the nuclear marker, 7-aminoactinomycin D, since it has useful properties (fluorescent dye) that would enable the isolation of cells in the image, as taught by Fraatz.

Further, the combination of Ortyn and Young and Fraatz collectively teach all of the claimed elements, the teaching of Fraatz performs the same function in combination with the combination of Ortyn and Young as taught individually in Fraatz, and the results

would be highly predictable (Identifying cell in the image using the fluorescent dye (7-aminoactinomycin D) as a nuclear maker).

C.) Claims 30-41 and 44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ortyn in view of Nicoletti et al. ("Common Methods for Measuring Apoptotic Cell Death by Flow Cytometry", 1997, The Purdue Cytometry CD-ROM Volume 3, Purdue University, West Lafayette, ISBN 1-890473-02-2, hereinafter Nicoletti).

As to Claim 44, Ortyn teaches the method of claim 29, wherein the step of collecting spatial frequency image data (**Ortyn, fig. 5; [0063], [0078-0080], [0135], "collection lens 32 is at about 90" angle relative to the directions of the light incident"**) comprises the steps of:

collecting a darkfield image of the specific cell (**Ortyn, fig. 5; [0135], darkfield image**); collecting a brightfield image of the specific cell (**Ortyn, fig. 5; [0135], brightfield image**); collecting a fluorescent image of the specific cell (**Ortyn, fig. 5; [0135], fluorescent image**); and wherein the step of analyzing comprises the step of determining that the specific cell is one of the following types of cells (**Ortyn, [0063], "spatial frequency content", [0135], identify "cell viability and apoptosis staging, and necrosis differentiation"; [0136], "cell analysis and classification"; [0138], "cell identification"**): a viable cell (**Ortyn, [0135], "cell viability"**); a cell that is in a relatively early stage of apoptosis (**Ortyn, [0135], "apoptosis staging,"**); a cell that is in a relatively late stage of apoptosis (**Ortyn, [0135], "apoptosis staging,"**); and a necrotic cell (**Ortyn, [0063], "necrosis differentiation"**). Ortyn provides an

apparatus/method for achieving the results of analysis, however is silent to what features separate the cells.

Enter, Nicoletti, who, like Ortyn, is directed to distinguishing cells into apoptotic cells, necrotic cells, based on morphologic features (**Nicoletti, see page 2, section a. Physical parameters of apoptotic cells, ¶1-2**). Specifically, Nicoletti teaches common methods that exploit physical parameters (cell size, granularity, cell density) and DNA content analysis (stainability) of cells to determine their viability (**Nicoletti, see page 2, section a., Physical parameters of apoptotic cells ¶1-2, page 3, section b. DNA content analysis ¶1., page 4, ¶1 - 2.**). Further, Nicoletti states the main advantages of this analysis is the simplicity, the low-cost and the possibility of its combination with other analysis (i.e. surface immunofluorescence) to identify the phenotype of apoptotic cells in a heterogeneous cell population.

Nicoletti and Ortyn are combinable because they are from the image arts of cell analysis. Thus, It would have been obvious to one of ordinary skilled in the art at the time of inventions looking at the teachings and suggestions of Ortyn to provide an improved the method of cell analysis of in view of Nicoletti's disclosure of using common methods that exploit physical parameters (cell size, granularity, cell density) and DNA content analysis (stainability) of cells to determine their viability. The modification to Ortyn, could be accomplished by including a combined method of using physical parameters (cell size, granularity, cell density) and DNA content analysis (stainability) of cells to determine their viability to distinguish the cells as (live, necrotic, early/late

apoptotic) to the method of Ortyn in view of the teachings of Nicoletti to obtain the invention as specified in the claim.

Further a person of ordinary skill in the art would have recognized the compatibility of using a combined method of using physical parameters (cell size, granularity, cell density) and DNA content analysis (stainability) of cells to determine their viability to distinguish the cells for the system of Ortyn. The combination has a reasonable expectation of success in that the modifications can be made using conventional and well known engineering and/or programming techniques, the methods taught by Nicoletti are not altered and continue to perform the same functions as separately, and the resultant combination produces the highly predictable result of using physical parameters (cell size, granularity, cell density) and DNA content analysis (stainability) of cells to determine their viability to distinguish the cells as (live, necrotic, early/late apoptotic). One of ordinary skilled in the art would have been motivated to combine the teachings of Nicoletti to the system/apparatus of Ortyn in order to use these features and method for distinguishing cells, given the simplicity, and low-cost for identifying cells in a heterogeneous cell population.

As to Claim 33, the combination of Ortyn and Nicoletti teach the method of claim 44 wherein the cell in the relatively early stare of apoptosis is characterized by a relatively smaller cellular area as determined from the brightfield image (**Nicoletti, page 2, ¶1, "Cells undergoing apoptosis display typical changes in their morphological and physical properties (cell shrinkage, condensation of chromatin and**

cytoplasm) which are well measurable by flow Cytometry", "Interaction of a particle with the laser beam produces a light scatter in a forward direction (FSC (*brightfield*), that correlates with cell size)") and no nuclear marker being present in the cell nucleus as determined by the fluorescent image (**Nicoletti, page 3, ¶4, page 4, ¶1-2, "The reduced stainability of apoptotic cells is the direct consequence of partial loss of DNA due to the activation of endogenous nucleases and diffusion of low-molecular weight DNA outside the cells"; "the presence of cells with DNA stainability lower than that of G1-cells has been considered a marker of cell death by apoptosis"**).

As to Claim 34, the combination of Ortyn and Nicoletti teach the method of claim 44 wherein the cell in the relatively early stare of apoptosis is characterized by a relatively higher scatter peak intensity as determined from the darkfield image (**Nicoletti, Fig.2, SSC (darkfield), page 2, ¶1, "Cells undergoing apoptosis display typical changes in their morphological and physical properties (cell shrinkage, condensation of chromatin and cytoplasm) which are well measurable by flow Cytometry", "Lateral direction (SSC, that correlates with granularity and/or cell density) "**) and no nuclear marker being present in the cell nucleus as determined by the fluorescent image (**Nicoletti, page 3, ¶4, page 4, ¶1-2, "The reduced stainability of apoptotic cells is the direct consequence of partial loss of DNA due to the activation of endogenous nucleases and diffusion of low-molecular weight DNA**

outside the cells"; "the presence of cells with DNA stainability lower than that of G1-cells has been considered a marker of cell death by apoptosis").

As to Claim 35 , the combination of Ortyn and Nicoletti teach the method of claim 44 wherein the cell in the relatively early stare of apoptosis is characterized by a relatively smaller cellular area as determined from the brightfield image (**Nicoletti, page 2, ¶1, "Cells undergoing apoptosis display typical changes in their morphological and physical properties (cell shrinkage, condensation of chromatin and cytoplasm) which are well measurable by flow Cytometry", "Interaction of a particle with the laser beam produces a light scatter in a forward direction (FSC (*brightfield*), that correlates with cell size)"**), a relatively higher scatter peak intensity as determined from the darkfield image (**Nicoletti, Fig.2, SSC (darkfield), page 2, ¶1, "Cells undergoing apoptosis display typical changes in their morphological and physical properties (cell shrinkage, condensation of chromatin and cytoplasm) which are well measurable by flow Cytometry", "Lateral direction (SSC, that correlates with granularity and/or cell density) "**), and no nuclear marker being present in the cell nucleus as determined by the fluorescent image (**Nicoletti, page 3, ¶4, page 4, ¶1-2, "The reduced stainability of apoptotic cells is the direct consequence of partial loss of DNA due to the activation of endogenous nucleases and diffusion of low-molecular weight DNA outside the cells"; "the presence of cells with DNA stainability lower than that of G1-cells has been considered a marker of cell death by apoptosis"**)..

As to Claim 36, the combination of Ortyn and Nicoletti teach the method of claim 44 wherein the cell in the relatively late stare of apoptosis is characterized by a relatively smaller cellular area as determined from the brightfield image (**Nicoletti, page 2, ¶1, "Cells undergoing apoptosis display typical changes in their morphological and physical properties (cell shrinkage, condensation of chromatin and cytoplasm) which are well measurable by flow Cytometry", "Interaction of a particle with the laser beam produces a light scatter in a forward direction (FSC (*brightfield*), that correlates with cell size)", page 3, ¶2, "In later stages of apoptosis, furthermore, the intensity of both FSC and SSC decreases due to the emerging secondary necrosis'**) and the nuclear marker being present in the cell nucleus as determined by the fluorescent image (**Nicoletti, page 2, ¶2-3, given the emerging secondary necrosis of later stages of apoptosis, one skilled in the art would expect to find traces of stained cellular DNA, given that necrotic cells take on stain**) .

As to Claim 37 the combination of Ortyn and Nicoletti teach the method of claim 44 wherein the cell in the relatively late stare of apoptosis is characterized by a relatively higher scatter peak intensity as determined from the darkfield image (**Nicoletti, page 2, ¶1, "Cells undergoing apoptosis display typical changes in their morphological and physical properties (cell shrinkage, condensation of chromatin and cytoplasm) which are well measurable by flow Cytometry", page 3,**

¶2, "In later stages of apoptosis, furthermore, the intensity of SSC decreases due to the emerging secondary necrosis' – however, examiner feels that one could still distinguish between necrotic and apoptosis based on the peak intensity, were necrotic would have a lower peak intensity – see figure 2.) and the nuclear marker being present in the cell nucleus as determined by the fluorescent image (**Nicoletti, page 2, ¶2-3, given the emerging secondary necrosis of later stages of apoptosis, one skilled in the art would expect to find traces of stained cellular DNA, given that necrotic cells take on stain**).

As to Claim 38 the combination of Ortyn and Nicoletti teach the method of claim 44 wherein the cell in the relatively late stare of apoptosis is characterized by a relatively smaller cellular area as determined from the brightfield image (**Nicoletti, page 2, ¶1, "Cells undergoing apoptosis display typical changes in their morphological and physical properties (cell shrinkage, condensation of chromatin and cytoplasm) which are well measurable by flow Cytometry", "Interaction of a particle with the laser beam produces a light scatter in a forward direction (FSC (*brightfield*), that correlates with cell size)", page 3, ¶2, "In later stages of apoptosis, furthermore, the intensity of both FSC decreases due to the emerging secondary necrosis'**), a relatively higher scatter peak intensity as determined from the darkfield image (**Nicoletti, page 2, ¶1, "Cells undergoing apoptosis display typical changes in their morphological and physical properties (cell shrinkage, condensation of chromatin and cytoplasm) which are well measurable by flow**

Cytometry", page 3, ¶2, "In later stages of apoptosis, furthermore, the intensity of SSC decreases due to the emerging secondary necrosis' – however, examiner feels that one could still distinguish between necrotic and apoptosis based on the peak intensity, were necrotic would have a lower peak intensity – see figure 2.), and the nuclear marker being present in the cell nucleus as determined by the fluorescent image (**Nicoletti, page 2, ¶2-3, given the emerging secondary necrosis of later stages of apoptosis, one skilled in the art would expect to find traces of stained cellular DNA, given that necrotic cells take on stain**).

As to Claim 39 the combination of Ortyn and Nicoletti teach the method of claim 44 wherein the necrotic cell is characterized by a relatively larger cellular area as determined from the brightfield image (**Nicoletti, page 2, ¶2-3, cellular size would increase in view of rupture of plasma membrane and leakage of cell content, thus it would be observable in FSC**) and the nuclear marker being present in the cell nucleus as determined by the fluorescent image (**Nicoletti, page 2, ¶2-3, necrotic cells take on stain**)..

As to Claim 40 the combination of Ortyn and Nicoletti teach the method of claim 44 wherein the necrotic cell is characterized by a relatively lower scatter peak intensity as determined from the darkfield image (**Nicoletti, page 2, ¶2-3, characterized by a**

reduction in both SSC) and the nuclear marker being present in the cell nucleus as determined by the fluorescent image (**Nicoletti, page 2, ¶2-3, necrotic cells take on stain**).

As to Claim 41 the combination of Ortyn and Nicoletti teach the method of claim 44 wherein the necrotic cell is characterized by a relatively larger cellular area as determined from the brightfield image (**Nicoletti, page 2, ¶2-3, cellular size would increase in view of rupture of plasma membrane and leakage of cell content, thus it would be observable in FSC**), a relatively lower scatter peak intensity as determined from the darkfield image (**Nicoletti, page 2, ¶2-3, characterized by a reduction in both SSC**), and the nuclear marker being present in the cell nucleus as determined by the fluorescent image (**Nicoletti, page 2, ¶2-3, necrotic cells take on stain**)..

As to Claim 30, the combination of Ortyn and Nicoletti teach the method of claim 44 wherein the viable cell is characterized by a relatively larger cellular area as determined from the brightfield image (**Nicoletti, page 2, ¶2-3, In view of the teachings of Nicoletti this is obvious/inherent given that a cells undergoing apoptosis (cell shrinkage, condensation of chromatin and cytoplasm) and cells necrotic death is characterized by a reduction in both FSC and SSC (probably due to a rupture of plasma membrane and leakage of the cell's content), so a viable cell what have a larger cellular area than say cells undergoing apoptosis – and**

visible in the FSC (brightfield) would displays the cell size) and no nuclear marker being present in the cell nucleus as determined by the fluorescent image (**Nicoletti, page 4, ¶2-3, page 5, ¶1. "Whereas the DNA content of unfixed apoptotic cells is unchanged compared to normal cells", "the difference in the DNA fluorescence of normal and apoptotic cells is minimal or undetectable" page3, ¶1-2, thus in view of the reduced stainability of apoptotic cells, one concludes the viable cells are also have a reduced stainability**).

As to Claim 31, the combination of Ortyn and Nicoletti teach the method of claim 44 wherein the viable cell is characterized by a relatively lower scatter peak intensity as determined from the darkfield image (**Nicoletti, Fig.2, page 2, ¶2-3, In view of the teachings of Nicoletti this is obvious/inherent to determine a viable cell based on its SSC (Darkfield) being lower as demonstrated in the figure, since during apoptosis there is an increase in SSC due to the chromatin condensation**) and no nuclear marker being present in the cell nucleus as determined by the fluorescent image (**Nicoletti, page 4, ¶2-3, page 5, ¶1. "Whereas the DNA content of unfixed apoptotic cells is unchanged compared to normal cells", "the difference in the DNA fluorescence of normal and apoptotic cells is minimal or undetectable" page3, ¶1-2, thus in view of the reduced stainability of apoptotic cells, one concludes the viable cells are also have a reduced stainability**).

As to Claim 32, the combination of Ortyn and Nicoletti teach the method of claim 44 wherein the viable cell is characterized by a relatively larger cellular area as determined from the brightfield image (**Nicoletti, page 2, ¶2-3, In view of the teachings of Nicoletti this is obvious/inherent given that a cells undergoing apoptosis (cell shrinkage, condensation of chromatin and cytoplasm) and cells necrotic death is characterized by a reduction in both FSC and SSC (probably due to a rupture of plasma membrane and leakage of the cell's content), so a viable cell what have a larger cellular area than say cells undergoing apoptosis – and visible in the FSC (brightfield) would displays the cell size**), a relatively lower scatter peak intensity as determined from the darkfield image (**Nicoletti, Fig.2, page 2, ¶2-3, In view of the teachings of Nicoletti this is obvious/inherent to determine a viable cell based on its SSC (Darkfield) being lower as demonstrated in the figure, since during apoptosis there is an increase in SSC due to the chromatin condensation**), and no nuclear marker being present in the cell nucleus as determined by the fluorescent image (**Nicoletti, page 4, ¶2-3, page 5, ¶1, "Whereas the DNA content of unfixed apoptotic cells is unchanged compared to normal cells", "the difference in the DNA fluorescence of normal and apoptotic cells is minimal or undetectable" page3, ¶1-2, thus in view of the reduced stainability of apoptotic cells, one concludes the viable cells are also have a reduced stainability**).

D.) Claims 42 and 43 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Ortyn and Nicoletti, further in view of Vitale et al. ("APOPTOSIS vs. NECROSIS", The Purdue Cytometry CD-ROM Volume 4, Publisher. Purdue University Cytometry Laboratories, West Lafayette, IN 1997, ISBN 1-890473-03-0, hereinafter Vitale).

As to Claim 42, the combination of Ortyn and Nicoletti teach the method of claim 44. However the combination is silent to wherein the step of analyzing the brightfield image comprises the step of determining if blebbing is present), blebbing being indicative of the cell in the relatively early stage of apoptosis and the cell in the relatively late stare of apoptosis, while lack of blebbing being indicative of the viable cell and the necrotic cell

Enter, Vitale, who, like Ortyn and Nicoletti, is directed to distinguishing cells into apoptotic cells, necrotic cells, based on morphologic features (**Vitale, see page 9, section 1. Introduction, APOPTOSIS vs. NECROSIS, ¶1**). Specifically, Vitale teaches surface blebbing is considered a pattern specific of apoptosis (**Vitale, see page 9, section 1. Introduction, APOPTOSIS vs. NECROSIS, ¶1**).

Vitale and the combination of Ortyn and Nicoletti are combinable because they are from the image arts of cell analysis. Thus, It would have been obvious to one of

ordinary skilled in the art at the time of inventions looking at the teachings and suggestions of Vitale to provide an method of detecting blebbing to the method of Ortyn and Nicoletti to further enhance cell identification.

Further a person of ordinary skill in the art would have recognized the compatibility of detecting blebbing to the method of using physical parameters (cell size, granularity, cell density) and DNA content analysis (stainability) of cells to determine their viability to distinguish the cells for the method of Ortyn and Nicoletti. The combination has a reasonable expectation of success in that the modifications can be made using conventional and well known engineering and/or programming techniques, the teachings of Vitale are not altered and continue to perform the same functions as separately, and the resultant combination produces the highly predictable result of using the physical parameters of blebbing to identify apoptosis.

As to Claim 43, the combination of Ortyn, Nicoletti, and Vitale teach the method of claim 42 wherein the step of analyzing the fluorescent image comprises the step of determining if the nuclear marker is present in the cellular nucleus, such that:

when no blebbing is determined to be present by analyzing the brightfield image (**Vitale, see page 9, section 1. Introduction, APOPTOSIS vs. NECROSIS, ¶1, blebbing is considered a pattern specific of apoptosis.**), and no nuclear marker is determined to be present in the cellular nucleus by analyzing the fluorescent image (**Nicoletti, page 4, ¶2-3, page 5, ¶1, "Whereas the DNA content of unfixed apoptotic cells is unchanged compared to normal cells", "the difference in the**

DNA fluorescence of normal and apoptotic cells is minimal or undetectable" page3, ¶1-2, thus in view of the reduced stainability of apoptotic cells, one concludes the viable cells are also have a reduced stainability), it can be concluded that the specific cell is the viable cell type (**Nicoletti, page 2, ¶2-3, In view of the teachings of Nicoletti this is obvious/inherent that a viable cell is identified by no blebbing, and also a reduced stainability**);

when blebbing is determined to be present by analyzing the brightfield image (**Vitale, see page 9, section 1. Introduction, APOPTOSIS vs. NECROSIS, ¶1, blebbing is considered a pattern specific of apoptosis – change in morphologic shape**), and no nuclear marker is determined to be present in the cellular nucleus by analyzing the fluorescent image, it can be concluded that the specific cell is the type of cell in a relatively early stage of apoptosis (**Nicoletti, page 2, ¶2-3, apoptosis has reduced stainability**);

when blebbing is determined to be present by analyzing the brightfield image (**Vitale, see page 9, section 1. Introduction, APOPTOSIS vs. NECROSIS, ¶1, blebbing is considered a pattern specific of apoptosis,**), and the nuclear marker is determined to be present in the cellular nucleus by analyzing the fluorescent image, it can be concluded that the specific cell is the type of cell in a relatively late stage of apoptosis (**Nicoletti, page 2, ¶2-3, given the emerging secondary necrosis of later stages of apoptosis, one skilled in the art would expect to find traces of stained cellular DNA, given that necrotic cells take on stain**); and when no blebbing is determined to be present by analyzing the brightfield image (**Vitale, see page 9,**

section 1. Introduction, APOPTOSIS vs. NECROSIS, ¶1, blebbing is considered a pattern specific of apoptosis,), and the nuclear marker is determined to be present in the cellular nucleus by analyzing the fluorescent image, it can be concluded that the specific cell is of the necrotic type of cell (**Nicoletti, page 2, ¶2-3, necrotic cells take on stain**).

E.) Claim 28 is further rejected under 35 U.S.C. 103(a) as being unpatentable over Ortyn as applied above in view of Fraatz.

As to Claim 28, Ortyn discloses the kit of claim 27. However, Ortyn doesn't explicitly teach wherein the single nuclear marker is 7-aminoactinomycin D.

Fraatz teaches using 7-aminoactinomycin D as a maker for imagining samples (**Fraatz, Column 8, Table 1, Table 2, Column 6, lines 1-20**). Fraatz performs analysis for identifying biological activities in specimens (cells). It would have been obvious to one of ordinary skilled in the art at the time of inventions to modify the method for identifying cells of Ortyn, by using 7-aminoactinomycin D as the nuclear marker as to the teaching of Fraatz. The combination of Ortyn and Fraatz are analogous in the art of image based biological analysis. One of ordinary skilled in the art would have been motivated to combine the teachings of Fraatz to the method of Ortyn in order to use the identify specific cells using the nuclear marker, 7-aminoactinomycin D, since it has

useful properties (fluorescent dye) that would enable the isolation of cells in the image, as taught by Fraatz.

Comment on 35 USC § 101

Independent claim 1, 8, 16, and 29 are in a "process" claim format and have been analyzed in light of *Bilski et al v. Kappos* [11], and the relevant guidance [2], [3]. The independent claims are not directed to an abstract idea at least because the independent claims encompasses more than just a statement of concept, and describes a particular solution to identifying cells using spatial frequency. Furthermore, the independent claim tangibly implements the method at least because a processor or equivalent hardware is necessary to perform the claimed "collecting/providing" and "analyzing/comparing" steps. Therefore, based upon consideration of all the relevant factors[3] with respect to the claims as a whole, claims 1, 8, 16, and 29 are not directed to an abstract idea.

Independent claim 27 is directed to a kit, which have been analyzed as an article claim (MPEP 2112.01). The claim recites a discrete physical material, specifically "a single nuclear marker." In view of the guidelines set out in MPEP 2106, the examiner concludes the claim and its dependents are statutory.

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[1] *See Bilski et al v. Kappos (SCt. 08-964),*

[2] *See Memorandum to the Examining Corps, Regarding the Supreme Court Decision in Bilski v. Kappos, issued June 28, 2010, available at http://www.uspto.gov/patents/law/exam/memoranda.jsp*

[3] *See Interim Guidance for Determining Subject Matter Eligibility for Process Claims in View of Bilski v. Kappos, Federal Registrar, Vol. 75, No. 143, issued July 27, 201*

Conclusion

This Office action has an attached requirement for information under 37 CFR 1.105. A complete reply to this Office action must include a complete reply to the attached requirement for information. The time period for reply to the attached requirement coincides with the time period for reply to this Office action.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

Vermes et al. "Flow cytometry of apoptotic cell death", Journal of Immunological Methods 243 (2000) 167–190, examiner refers applicant to abstract, fig.1, and page 182

Darzynkiewicz et al. Features of Apoptotic Cells Measured by Flow Cytometry, 1992, Cytometry 13, pages 795-808

Contact

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jason Heidemann:

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The examiner can normally be reached on Monday - Thursday/7:30 A.M. to 5:00 P.M.. For e-mail communications, please note MPEP 502.03, which states, in relevant part, "[w]ithout a written authorization by applicant in place, the USPTO will not respond via Internet e-mail to any Internet correspondence which contains information subject to the confidentiality requirement as set forth in 35 U.S.C. § 122." A sample authorization form which may be used by applicant can be found in MPEP 502.03 section II.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Matthew Bella can be reached on 571-272-7778. The fax phone numbers for the organization where this application or proceeding is assigned are 571-273-8300 for regular communications and 571-273-8300 for After Final communications. TC 2600's customer service number is 571-272-2600.

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